Progress in Neuro-Psychopharmacology & Biological Psychiatry 38 (2012) 276–284

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

The anxiolytic-like effect of an essential oil derived from *Spiranthera odoratissima* A. St. Hil. leaves and its major component, β-caryophyllene, in male mice

Pablinny Moreira Galdino [a,b,*], Marcus Vinícius Mariano Nascimento [a], Iziara Ferreira Florentino [a], Roberta Campos Lino [a], James Oluwagbamigbe Fajemiroye [a], Beatriz Abdallah Chaibub [c], José Realino de Paula [c], Thereza Christina Monteiro de Lima [b], Elson Alves Costa [a]

[a] Laboratório de Farmacologia de Produtos Naturais, Instituto de Ciências Fisiológicas, Universidade Federal de Goiás, Goiânia, GO, Brazil
[b] Laboratório de Neurofarmacologia, Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
[c] Laboratório de Pesquisa em Produtos Naturais, Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil

ARTICLE INFO

Article history:
Received 17 December 2011
Received in revised form 10 April 2012
Accepted 14 April 2012
Available online 21 April 2012

Keywords:
Anxiolytic-like effect
β-caryophyllene
Flumazenil
NAN-190
Spiranthera odoratissima

ABSTRACT

Spiranthera odoratissima A. St. Hil. (manacá) is used in folk medicine to treat renal and hepatic diseases, stomachache, headaches and rheumatism. A central nervous system (CNS) depressant effect of the hexane fraction from the ethanolic extract of this plant has been described. β-caryophyllene, the main component of this essential oil, is a sesquiterpene compound with anti-inflammatory properties that has been found in essential oils derived from several medicinal plants.

This work is aimed to evaluate the pharmacological activity of the essential oil obtained from *S. odoratissima* leaves (EO) and its major component on the murine CNS; we aimed to evaluate a possible anxiolytic-like effect and the underlying mechanisms involved. In an open field test, EO (500 mg/kg) and β-caryophyllene (50, 100 and 200 mg/kg) increased the crossing frequency ($P<0.05$) and, EO (250 and 500 mg/kg) and β-caryophyllene (200 mg/kg) increased the time spent in the center ($P<0.05$) without altering total crossings of the open field. EO and β-caryophyllene did not alter the number of falls in the rota-rod test ($P>0.05$). In the pentobarbital-induced sleep test, EO (500 mg/kg) and β-caryophyllene (200 and 400 mg/kg) decreased the latency to sleep ($P<0.05$), and EO (125, 250 and 500 mg/kg) ($P<0.001$) and β-caryophyllene (200 and 400 mg/kg) ($P<0.05$ and $P<0.001$) increased the sleep time. In anxiety tests, EO (500 mg/kg) and β-caryophyllene (100 and 200 mg/kg) increased head-dipping behavior ($P<0.05$) in the hole-board test, entries ($P<0.05$) into and time spent ($P<0.05$) on the open arms of the elevated plus maze (EPM), and number of transitions ($P<0.05$) and time spent in the light compartment ($P<0.05$) of a light–dark box (LDB). We further investigated the mechanism of action underlying the anxiolytic-like effect of EO and β-caryophyllene by pre-treating animals with antagonists of benzodiazepine (flumazenil) and 5-HT_{1A} (NAN-190) receptors prior to evaluation using EPM and LDB. The anxiolytic-like effects of EO were significantly reduced by pre-treatment with NAN-190 ($P<0.05$) but not flumazenil ($P>0.05$). The anxiolytic-like effects of β-caryophyllene were not blocked by either NAN-190 or flumazenil ($P>0.05$). In conclusion, these results suggest that the essential oil derived from *S. odoratissima* produces an anxiolytic-like effect without altering motor performance and that this effect is mediated by 5-HT_{1A} but not via benzodiazepine receptors. In addition, the major component, β-caryophyllene, also has an anxiolytic-like effect that may contribute to the effects of EO, but this effect does not seem to be mediated via 5-HT_{1A} or benzodiazepine receptors.

1. Introduction

Anxiety and stress disorders are among the most common of all chronic diseases. The prevalences of these disorders are increasing in many countries, and these disorders have a much earlier age of onset than other chronic conditions (Kessler and Greenberg, 2002). As an example, in Brasilia city (the capital of Brazil), anxiety disorders affect 12.1% of the population and are the most commonly diagnosed psychiatric disorders (Almeida Filho et al., 1992).

Since the introduction of benzodiazepines in the 1960s, they have been the most commonly prescribed treatment for anxiety, remaining

Abbreviations: CNS, central nervous system; EO, essential oil obtained from *S. odoratissima* leaves; EPM, elevated plus maze test; LDB, light–dark box test; UFG, Universidade Federal de Goiás; NAN-190, 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido) butyl] piperazine hydrobromide; p.o., orally; i.p., intraperitoneally; s.c., subcutaneously; SEM, standard error of the mean; GAD, generalized anxiety disorder; CB1, cannabinoid 1 receptor; CB2, cannabinoid 2 receptor; 5-HT_{1A} receptor, 5-hydroxytryptamine 1A receptor.

* Corresponding author at: Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ciências Fisiológicas, CP 131, 74001-970, Goiânia, GO, Brazil. Tel.: +55 62 35211491; fax: +55 62 3521 1204.
E-mail address: pablinnyg@yahoo.com.br (P.M. Galdino).

0278-5846/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2012.04.012

the mainstay of pharmacological treatment in anxiety disorders. However, they have prominent side effects, such as sedation, myorelaxation, ataxia, and amnesia, and they can cause pharmacological dependence (Lader and Morton, 1991). Thus, new therapies for the treatment of anxiety disorders are necessary, and the study of medicinal plants could provide new therapeutic options (Faustino et al., 2010).

The anxiolytic-like effect of plants from Rutaceae family has already been demonstrated; such plants include *Citrus aurantium* L. (Carvalho-Freitas and Costa, 2002), *Casimiroa edulis* La Llave ex Lex. (Molina-Hernández et al., 2004; Mora et al., 2005) and *Ruta chalepensis* L. (Gonzalez-Trujano et al., 2006).

Spiranthera odoratissima A. St. Hillaire (Rutaceae) is a shrub that is found in the Brazilian Cerrado region and popularly known in Brazil as *manacá*. Its flowers are whitish and very aromatic, its fruits possess a unique seed and its roots are woody, with yellow-gold coloration (Almeida et al., 1998). In folk medicine, its leaves are used as a blood purgative and in the treatment of renal and hepatic diseases (Salles et al., 1997), while the roots are used to as an appetite stimulant and to treat stomachache, headaches, sore muscles, and hepatic dysfunction (Silva, 1998). In the state of Goiás, Brazil, these roots are also used to treat rheumatism (Tresvenzol et al., 2006).

According to Matos et al. (2003), the aqueous fraction from the ethanolic extract of manacá leaves shows analgesic and anti-inflammatory activities in the acetic acid-induced writhing, croton oil-induced ear edema and carrageenan-induced peritonitis models. Similar results were obtained with the ethanolic extract of manacá roots, which are also characterized by the central nervous system depressant activity (Matos et al., 2004). The evaluation of the effects of the hexane fraction from the ethanolic extract of *S. odoratissima* leaves on the murine CNS indicates that this fraction has active substances that increase the sleep time in a pentobarbital-induced sleep test (Matos et al., 2006).

β-Caryophyllene, the major component of *S. odoratissima* essential oil (Chaibub and Paula, 2009), is a sesquiterpene compound found in the essential oils of many different species (Gertsch et al., 2008; Molina-Jasso et al., 2009). Among the pharmacological activities of this compound are anti-inflammatory (Fernandes et al., 2007; Medeiros et al., 2007; Passos et al., 2006) and antispasmodic activities (Leonhardt et al., 2010); it has also been used as a local anesthetic (Ghelardini et al., 2001) and gastric cytoprotector (Tambe et al., 1996), to stimulate natural killer cells (Standen et al., 2006). It has also been shown to be neuroprotective in human neuroblastoma (Chang et al., 2007).

Then, this work is aimed to evaluate the neuropharmacological activity of the essential oil from *S. odoratissima* leaves in the CNS, with special attention to possible anxiolytic-like effects, as well as the roles of $5HT_{1A}$ and $GABA_A$/benzodiazepine receptors in these effects. The identification of the active component(s) is one step in the proposed development of an herbal drug; therefore, this paper includes examination of the anxiolytic-like effect and possible mechanism of action of the essential oil's major compound, β-caryophyllene.

2. Material and methods

2.1. Animals

Male adult Swiss mice weighing approximately 30 g (n = 765) were used in all experiments. All animals were used only once. The animals were provided by the Central Animal House of Federal University of Goiás (Universidade Federal de Goiás — UFG); they were housed in groups of 20 mice/cage and were kept in a room with controlled temperature (25 ± 1 °C) and lighting (light/dark cycle of 12 h, lights on at 7 am), with food and water ad libitum. The animals were kept in the laboratory for an adaptation period of at least 1 h before the experiments. All experimental protocols were developed in accordance with the principles of ethics and animal welfare recommended by Brazilian Science Society of Laboratory Animal (Sociedade Brasileira de Ciência em Animais de Laboratório) and were approved by the UFG Institutional Ethical Committee (Protocol 104/2008).

2.2. Plant material and essential oil extraction

S. odoratissima A. St.-Hil. leaves were collected in December 2007 near the town of Senador Canedo, Goiás, Brazil (762 m, 16°45′45.2″ S, 49°07′06.8″ W) and were authenticated by Prof. Dr. José Realino de Paula (Pharmacy Faculty — UFG). A voucher specimen was deposited at the Herbarium of the UFG (UFG — 30,275). The leaves were dried at room temperature and crushed, and the essential oil (EO) was extracted by hydrodistillation for approximately 3 h using a Clavenger-type apparatus, with a yield of 2.3%. The EO was subjected to gas chromatographic mass spectrometry in SHIMADZU QP5050A equipment, and the chemical components were identified by comparing the mass spectra and the retention indexes with the literature. The major components identified were β-caryophyllene (20.64%), gamma-muurolene (17.7%), bicyclogermacrene (14.73%), delta-cadinene (13.40%), gamma-cadinene (4.59%) and cubenol (3.12%) (Chaibub and Paula, 2009).

2.3. Drugs

EO and β-caryophyllene (Sigma — Brazil) were emulsified with 2% Tween 80 (Sigma — USA) and dissolved in distilled water. Sodium pentobarbital (Abbott — Brazil) was dissolved in saline. Diazepam (Cristália — Brazil) was dissolved in distilled water. It is well known that benzodiazepines act as anxiolytics at low doses and that they induce sedation and myorelaxant effects at higher doses (Novas et al., 1988). Therefore, we used diazepam (1 mg/kg) as a positive control for anxiolytic-like effects, and diazepam (5 mg/kg) as a positive control for sedative and myorelaxant effects. Flumazenil (União Química — Brazil) and NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido) butyl] piperazine hydrobromide — Sigma — USA) were used as pretreatments to verify the role of $GABA_A$/benzodiazepine and $5\text{-}HT_{1A}$ receptors, respectively.

2.4. Behavioral procedures

2.4.1. General behavior test

Experimental groups of mice (n = 5 per group) were treated orally (p.o.), intraperitoneally (i.p.) or subcutaneously (s.c.) with OE at doses of 10, 30, 100, 300 or 1000 mg/kg or with β-caryophyllene at doses of 50, 100 or 200 mg/kg, whereas control groups received vehicle (2% Tween 80 in distilled water, 10 mL/kg) by the same routes. The animals were observed in free ambulation on a flat surface for 3 min, again at 5, 10, 20, 30 and 60 min, and 4, 8, 24 and 48 h after the treatments and after 4 and 7 days of treatment. The observed effects were noted using a standard pharmacological screening approach, adapted from Malone (1977).

2.4.2. Rota-rod test

In this test, the animals were pre-selected in a training session 24 h before the test based on their ability to remain on the bar (at 12 rpm) for 2 min. Groups of pre-selected animals (n = 9) were treated (p.o.) with vehicle (2% Tween 80, 10 mL/kg), EO (125, 250 or 500 mg/kg), β-caryophyllene (50, 100, 200 or 400 mg/kg) or diazepam (1 or 5 mg/kg). Sixty minutes after the treatment, the animals were placed with all four paws onto the bar and the number of falls was evaluated. The maximum time allowed was 1 min, and the maximum number of falls allowed was three (Dunham and Miya, 1957).

2.4.3. Open-field test

Experimental groups of 9 mice were treated (p.o.) with vehicle (2% Tween 80, 10 mL/kg), EO (125, 250 or 500 mg/kg), β-caryophyllene (50, 100 or 200 mg/kg) or diazepam (1 or 5 mg/kg). Sixty minutes